SUPPLEMENTARY METHODS:

Genotype analysis

We performed OMNI Express high-resolution cSNP arrays (~720K) for families A (IV.1, III.1, III.2) and E (II.1, I.1., I.2). We used the data to obtain the length of the haplotype block around the causative COPA mutations shared by affected individuals in two families. For each family we phased and compared the genotype calls for each trio to identify haplotypes inherited from by affected family members. The haplotype analysis of cSNP data was performed using custom scripts implemented in R Statistical Programming Language.

Autophagy Stimulation and Detection

Functional and biochemical analyses were performed using the following reagents: Torin1 (EMD Millipore Chemicals); Baflomycin A1 (Sigma-Aldrich); Guinea pig anti-p62 C terminal antibody (American Research Product, INC); Rabbit anti-LC3b antibody (Novus Biologicals); Rabbit phospho-S6 ribosomal protein Ser235/236 antibody (Cell Signaling); S6 ribosomal protein (54D2) mouse monoclonal antibody (Cell Signaling); Mouse monoclonal anti-GAPDH antibody (Sigma-Aldrich); Infrared fluorescent dyes conjugated secondary antibodies (Li-Cor). To perform the experiments, an mTOR specific inhibitor Torin1 was used to induce autophagy, and a lysosome inhibitor Bafilomycin A were used to block the degradation of autophagy content by lysosome. Briefly, cells ($2x10^6$) were treated with vehicle only, 250nM Torin1 or 250nM Torin1 together with 200nM Bafilomycin A for 4h. Cells were washed twice with phosphate-buffered saline and lysed on ice for 30 min in GFB buffer (40mM Bis-tris propane, 150 mM NaCl, 1 % Triton X-100, 10% Glycerol) supplemented with 1x PIC (protease inhibitor cocktail, BD biosciences) and 1x Phosphatase Inhibitor Cocktail (Thermo Scientific). After centrifugation at 5,000g for 1min, protein concentrations in cell lysate supernatants were measured using BCA protein assay reagents (Thermo Scientific). Cell lysates (25 µg protein) were then heated at 95°C for 5 min in 1x LDS sample buffer (Invitrogen) and resolved by SDS polyacrylamide gel electrophoresis (PAGE) (Invitrogen). Proteins were transferred to nitrocellulose membranes by using a wet transfer cell system (Invitrogen). Membranes were blocked with blocking buffer (Li-Cor) for 1 h, followed by a 2-h incubation with primary antibody and 1-h incubation of secondary antibody in block buffer supplemented with 0.1% Tween-20. Immunoreactive bands were visualized with using Odyssey Infrared Imaging System (Li-Cor). For direct assessments of autophagosomes, cells were allowed to adhere to poly L-Lysine coated coverslips for 20 min at 37°C, 5% CO_2, which were then washed in PBS and permeabilized with Cytofix/Cytoperm reagent (BD). Cells were then stained with rabbit IgG αLC3B clone D11 (Cell Signaling Technologies) and mouse IgG αCD107a clone H4A3 (BD Biosciences). Cells were washed and then stained with α-Mouse Alexafluor-488 and α-Rabbit Alexafluor-532. GFP-LC3 expressing HEK293T cells were electroporated as stated above. Cells were imaged as stated below. GFP puncta formation was quantified to indicate autophagosome formation upon expression of mutant or wild type COPA protein.

Microscopy

For Stimulation Emission Depletion (STED) microscopy cells were allowed to adhere to poly L-Lysine coated coverslips for 20 min at 37°C, 5% CO_2, which were then washed in PBS and permeabilized with Cytofix/Cytoperm reagent (BD). Cells were then stained with rabbit IgG αLC3B clone D11 (Cell Signaling Technologies) and mouse IgG αCD107a clone H4A3 (BD Biosciences) for 1 hour. Cells were washed and then stained with Alexa Fluor 488 Goat anti-Mouse IgG and Alexa Fluor 532 Goat anti-Rabbit (Life Technologies) for 30 minutes. Slides were then visualized on Leica SP8 TCS STED microscope (Leica Microsystems). Acquisition was performed as previously described.23 Briefly, excitation was performed using a tunable white light laser and emission was detected by HyD detectors. Settings were adjusted based on single color controls and independent channels were acquired sequentially as to limit fluorescence contamination. Images were acquired using LAS AF (Leica) and exported to Volocity software (PerkinElmer) for analysis. For confocal microscopy GFP-LC3 expressing HEK293T cells were electroporated as stated above. Electroporated cells were plated on a delta T imaging chamber (Bioptechs Inc.) and 24 hours later cells were imaged by confocal microscopy on a Zeiss Axio Observer Z1 with Yokogawa CSU10 spinning disk. Images were acquired with a Hamamatsu Orca-AG camera using Volocity Software (PerkinElmer). Excitation was performed using a 488nm laser with optimized exposure times. Images were analyzed using Volocity software.

For COPA staining and co-staining with the ERGIC compartment in BLCL, cytospins were performed. After fixing with 0.2% Triton X-100, 2% Formaldehyde in PBS and blocking, cells were stained with anti-COPA (Sigma), anti-ERGIC-53 (Santa Cruz) and DAPI. Staining was visualized by using a Zeiss Apotome widefield microscope.

Image Analysis

Microscopy images were analyzed using Volocity software. For confocal images intensity of GFP was thresholded based on 2 standard deviations above the mean intensity. Objects under 1 μm^3 were excluded. For STED images a signal to noise ratio of 2 was used to define positive signal. Objects smaller than 0.1 μm^2 were excluded from analysis. All values were exported to GraphPad 6.0 (Prism Software) for graphing and statistical analysis. Presented images were contrast enhanced uniformly.

Supplementary Table 1: Demographics and clinical information of patients[✓]									
PATIENTS	**A.III.6**	**A.IV.1**	**B.III.1**	**C.IV.1**	**C.IV.2**	**D.I.2**	**D.III.5**	**E.I.2**	**E.II.1**
Demographics & initial symptoms									
Current age	20	14	20	33	20	67	10	35	6
Sex	F	M	F	F	F	F	F	F	F
Race	White	White	White	White	White	White	White	Asian	Asian
Age of Clinical Presentation	4 years	2 years	6 months	2 years	4 years	unknown	7 years	5 years	2 years
Symptoms at presentation	joint pain, shortness of breath	cough, anemia	cough	joint pain	joint pain	unknown	lethargy, abdominal pain	tachypnea	joint pain, tachypnea
Features of Systemic Inflammation									
CRP (mg/L)	<5.0- 10.1	0.5	NA	16.4-91.9	8.2-12.7	NA	<0.6	4.7	10.9
ESR (mm/hr)	46-123	26	NA	38-68	22-53	NA	<10	40	47
Clinical Features Pulmonary Disease									
Interstitial Lung Disease	yes	yes	yes	yes	yes	yes	yes	yes	yes
Pulmonary Hemorrhage	yes	yes	yes	yes	no	yes	yes	yes	no
Lung restriction	yes	yes	yes	yes	yes	yes	yes	yes	NA
Lung obstruction	yes	no	no	yes	yes	yes	NA	yes	NA
Diffusion capacity defect	NA	no	NA	yes	yes	yes	NA	yes	NA
Clinical Features of Joint Disease									
Signs or symptoms of arthritis	yes	yes	yes	yes	yes	NA	no	yes	yes
erosive or non-erosive	avascular necrosis, s/p knee replacement	NA	non-erosive	non-erosive	non-erosive	NA	--	non-erosive	non-erosive
Clinical Features of Renal Disease									
Renal Biopsy	immune complex crescentic glomer-ulonephritis with fibrosis and fibrocellular crescents	--	Arteriolar C3 & C4; focal mesangial hypercellularity with faint arteriolar C3 & tubuloreticular inclusions	--	--	--	IgA nephropathy with necrotizing lesions	focal segmental sclerosing glomer-ulonephritis with fibrosis and fibrocellular crescents	--
Other Clinical Features	Autoimmune thyroid disease	--	--	--	--	Paroxysmal exertional dyskin-esia; leukocytoclas-tic angiitis†	Paroxysmal exertional dyskinesia†	--	--
Immunomodulatory Treatments	Pre-kidney transplant: cyclophosphamide, steroids*, azathio-prine*. Post: etanercept*, cyclosporine*, plaquinel*	steroids, methotrexate, etanercept*	cyclophosphamide*, steroids^, azathioprine^	steroids*, mycophenolate*, cyclophosphamide^	steroids*, mycophenolate*	NA	cyclophosphamide*, steroids*, azathioprine[#]	cyclophosphamide*, prednsione*, azathioprine*	prednisone*, mycophenolate*, plaquenil*, rituximab[#]

✓Demographics and expanded clinical information for representative patients of Families A-E. The term NA denotes not available. †All affected carriers in Family D have paroxysmal exertional dyskinesia of unclear etiology and do not have mutations in *GLUT1*; no other family has neurologic symptoms or disease. *responded to treatment, ^partial response to treatment, #treatment failure

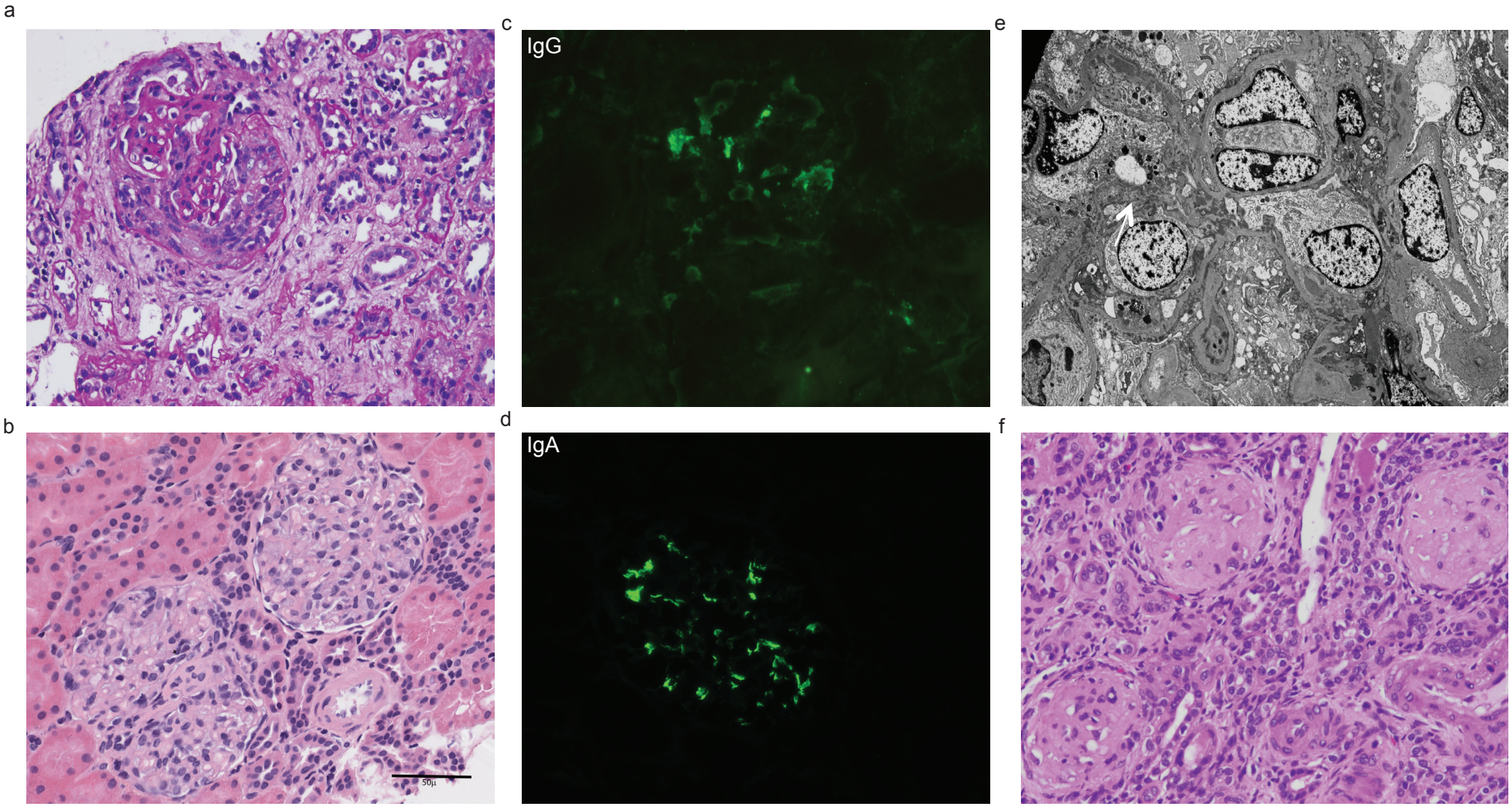
Supplementary Table 2: Autoantibodies										
	A.III.6	**A.IV.1**	**B.III.1**	**C.IV.1**	**C.IV.2**	**C.V.1**	**C.V.9**	**D.III.5**	**E.I.2**	**E.II.1**
ANA (<1:40)	1:1280	1:1280	1:160	+^	1:640	–	+^	–	1:1280	1:640
ANA pattern	homogenous	homogenous	homogenous	speckled	diffuse	NA	homogenous	NA	homogenous	speckled
ANCA (negative)	+	–	–	+	+	+*	+	+	+	+
MPO (<4 EU/mL)	21.4	–	–	46.6	–	–	311	15	NA	NA
PR3 (<4 EU/mL)	–	–	–	–	21.8	–	–	–	NA	NA
Sm antibody (<0.8)	–	–	–	–	–	NA	NA	–	–	–
Sm/RNP antibody (<0.8)	–	–	–	–	–	NA	–	–	–	8.7#
SSA antibody (<0.8)	–	–	–	–	–	NA	–	–	NA	–
SSB antibody (<0.8)	–	–	–	–	–	NA	–	–	NA	–
RF (<40 IU/mL)	>320	92	–	–	–	NA	–	320	–	–
anti-CCP (<20 U)	NA	>250	–	–	–	NA	–	NA	NA	NA
dsDNA (<25 IU/mL or titer)	–	–	+ 1:40	–	–	NA	–	–	+	–

Normal values for each test indicated in parentheses. + or – refers to positive or negative test result, *perinuclear, ^no titer available, #equivocal, ANCA anti neutrophil cytoplasmic antibody, NA not available, RF rheumatoid factor. Of note, both the ANCA and ANA levels wax and wane with treatment.

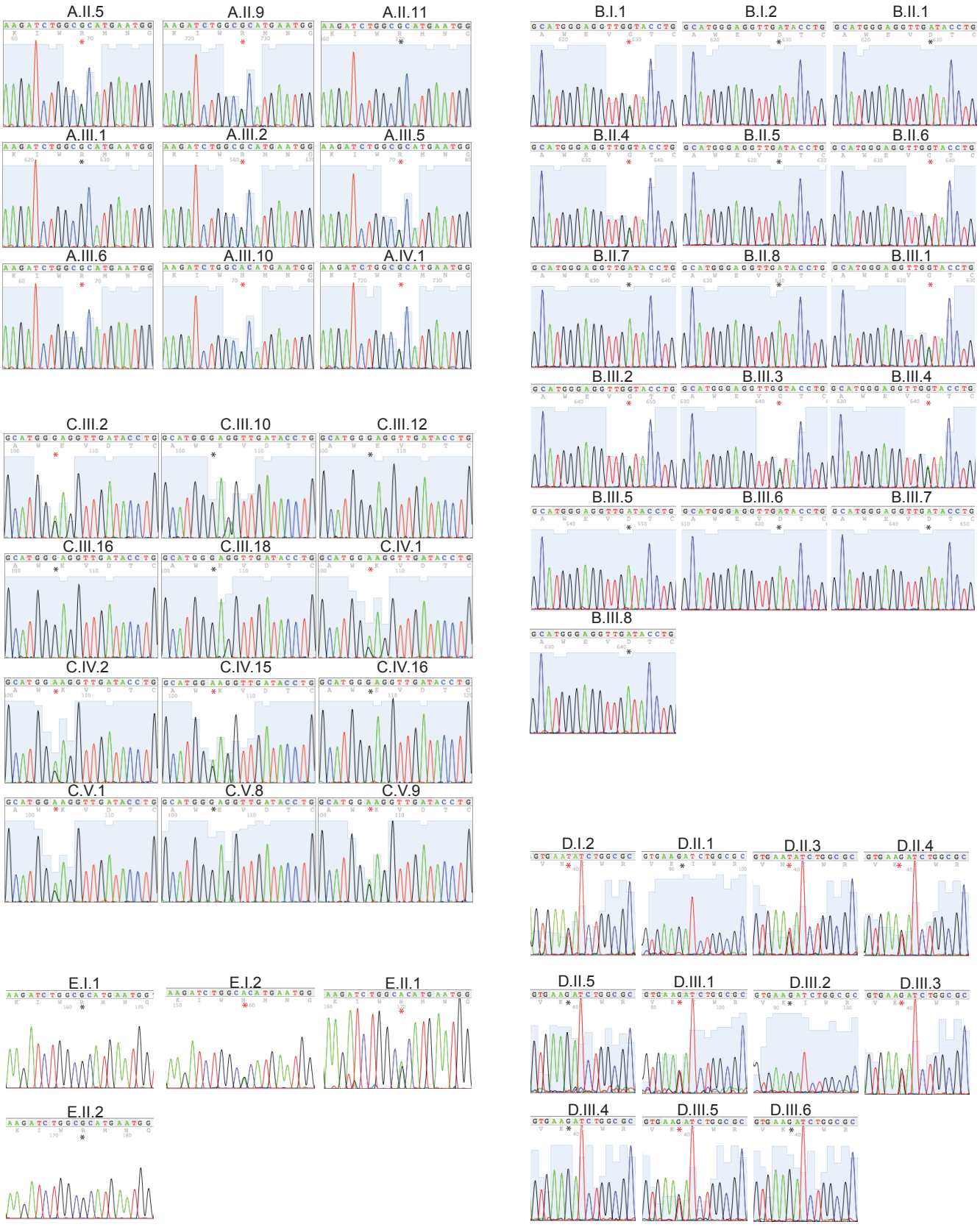
Supplementary Table 3: Immunophenotyping Data						
	A.III.6	**A.IV.1**	**B.III.1**	**C.IV.1**	**C.IV.2**	**E.II.1**
Immunoglobulins						
IgG mg/dL	479 (641-1353)	2291 (893-1823)	582 (631-1353)	2000 (672-1760)	3200 (672-1760)	2360 (320-1150)
IgA mg/dL	100 (66-295)	222 (70-432)	61 (66-295)	247 (89-581)	650 (89-581)	170 (30-90)
IgM mg/dL	218 (40-180)	95 (52-367)	97 (40-180)	82 (39-333)	45 (39-333)	300 (50-190)
Immune cell subsets						
Absolute lymphocyte count	1207 (950-3527)	1866 (1200-5200)	NA	3693 (870-3790)	3722 (870-3790)	3863 (1200-7100)
CD3 T cells %	92% (58-88)	86% (61-82)	NA	75% (55-84)	59% (55-84)	62% (43-76)
CD3 T cells absolute	1110 (798-2594)	1183 (860-2420)	NA	2715 (690-2540)	2218 (690-2540)	2432 (900-4500)
CD4 T cells %	54% (30-63)	NA	NA	45% (31-60)	36% (31-60)	39% (23-48)
CD4 T cells absolute	657 (579-1841)	NA	NA	1629 (410-1590)	1354 (410-1590)	1521 (500-2400)
CD4 CD8 ratio	1.5 (1.1-3.5)	NA	NA	1.7 (0.8-4.2)	1.6 (0.8-4.2)	2.0 (0.9-2.9)
CD8 T cells %	37% (11-39)	NA	NA	27% (13-41)	23% (13-41)	20% (14-33)
CD8 T cells absolute	447 (184-855)	NA	NA	977 (190-1140)	865 (190-1140)	762 (300-1600)
CD19 B cells %	0%* (3-25)	9 (9-30)	NA	23% (6-25)	24% (6-25)	30% (14-44)
CD19 B cells absolute	0* (63-461)	120 (130-800)	NA	833 (90-660)	902 (90-660)	1165 (200-2100)
CD16, CD56 NK Cells %	8% (3-22)	NA	NA	4% (5-27)	16% (5-27)	7% (4-23%)
CD16, CD56 NK Cells absolute	97 (89-472)	NA	NA	145 (90-590)	602 H (90-590)	262 (100-1000)

Normal ranges shown in parentheses. Immune cell subsets expressed in $x10^6$ cells/L. *Patient previously treated with rituximab; NA not available

Supplemental Figure 1



Supplemental Figure 1. **Renal Pathology of patient A.III.6 (a,c,e-f) and D.III.5 (b,d). (a)** Renal biopsy H&E staining shows immune complex crescentic glomerulonephritis with fibrocellular crescents **(b)** and glomeruli with mild mesangial expansion and hypercellularity. **(c)** IgG+ and **(d)** IgA+ mesangial foci confirm antibody deposits. **(e)** Electron micrograph reveals rare electron dense deposits. **(f)** Kidney explant H&E staining shows end-stage renal disease with diffuse global sclerosis and interstitial chronic inflammation.



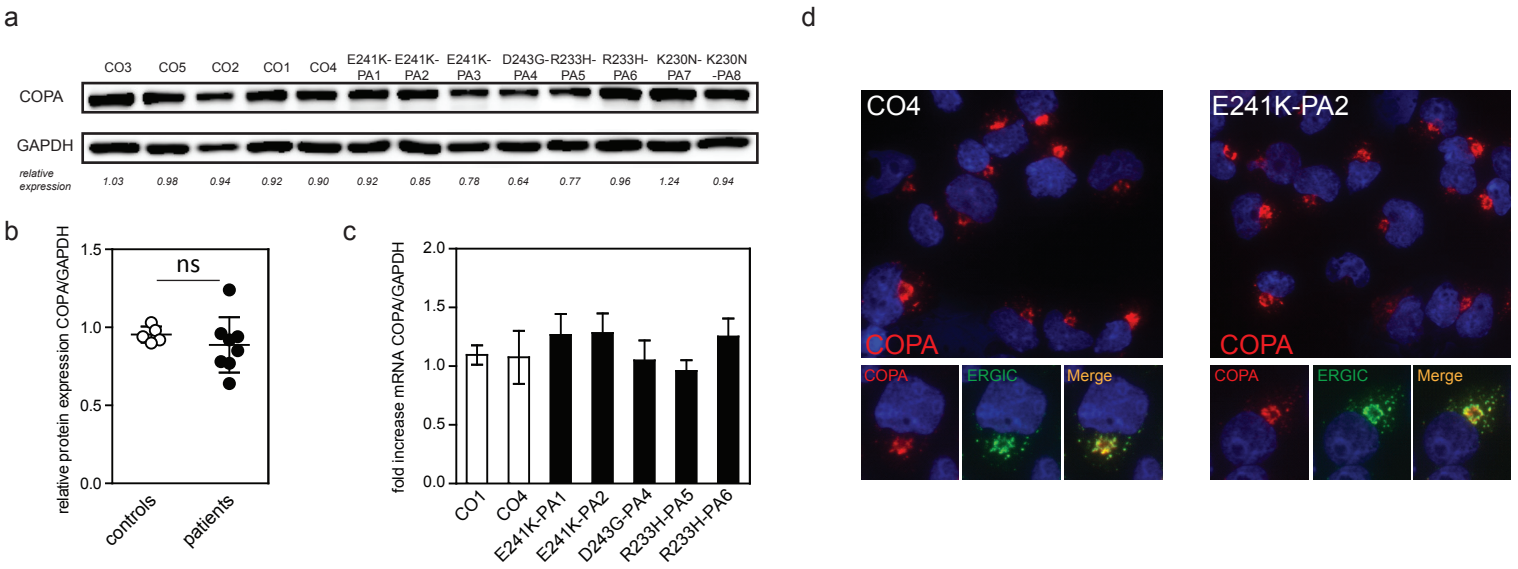
Supplementary Figure 2. Sanger sequencing chromatograms for *COPA* in family members. Sanger sequencing reactions were performed on genomic DNA covering the area identified by whole exome sequencing with a 20 bp snapshot around the point mutation shown. * Indicates mutation position. Red * indicates mutation. Patient identifiers are shown above chromatograms.

Supplementary Table 4. Results of haplotype analysis in *COPA* locus*

SNP	Haplotype 1	Haplotype 2
rs659235	G	A
rs617698	A	A
rs3747623	G	G
rs6697826	A	G
rs2795070	G	G
rs16831591	A	A
rs4656252	G	G
rs16831606	G	G
rs1132881	G	G
rs16831619	G	G
rs1061511	A	A
rs11265359	G	G
rs16831634	G	G
rs16831675	A	A
rs11265362	G	G
rs2275700	G	G
rs6683177	G	G
rs7554737	A	A
rs1557524	A	A
rs9853	C	C
rs11265368	A	A
rs16831746	A	A
rs1317944	A	A
rs2066320	G	G
rs12060052	G	G
rs7522166	G	G
rs1802778	A	A
rs10752637	C	C
rs1324738	G	G
rs6669689	U	G
rs16831846	A	A
rs12239747	A	A
rs6664438	G	G
rs1124379	A	G
rs6677637	G	G
rs12724113	G	G
rs11265374	A	A

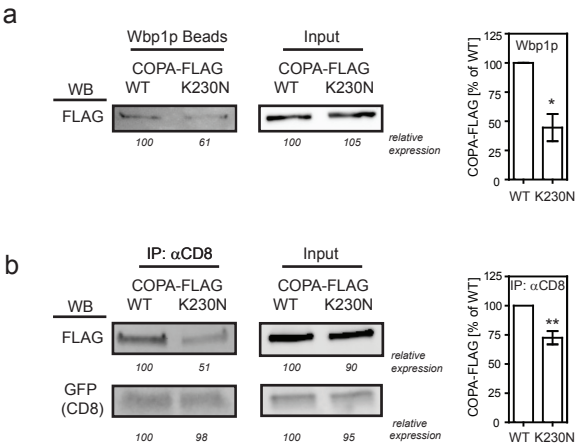
* shared haplotype marked in light grey, SNPs within COPA marked in dark grey

Supplementary Figure 3



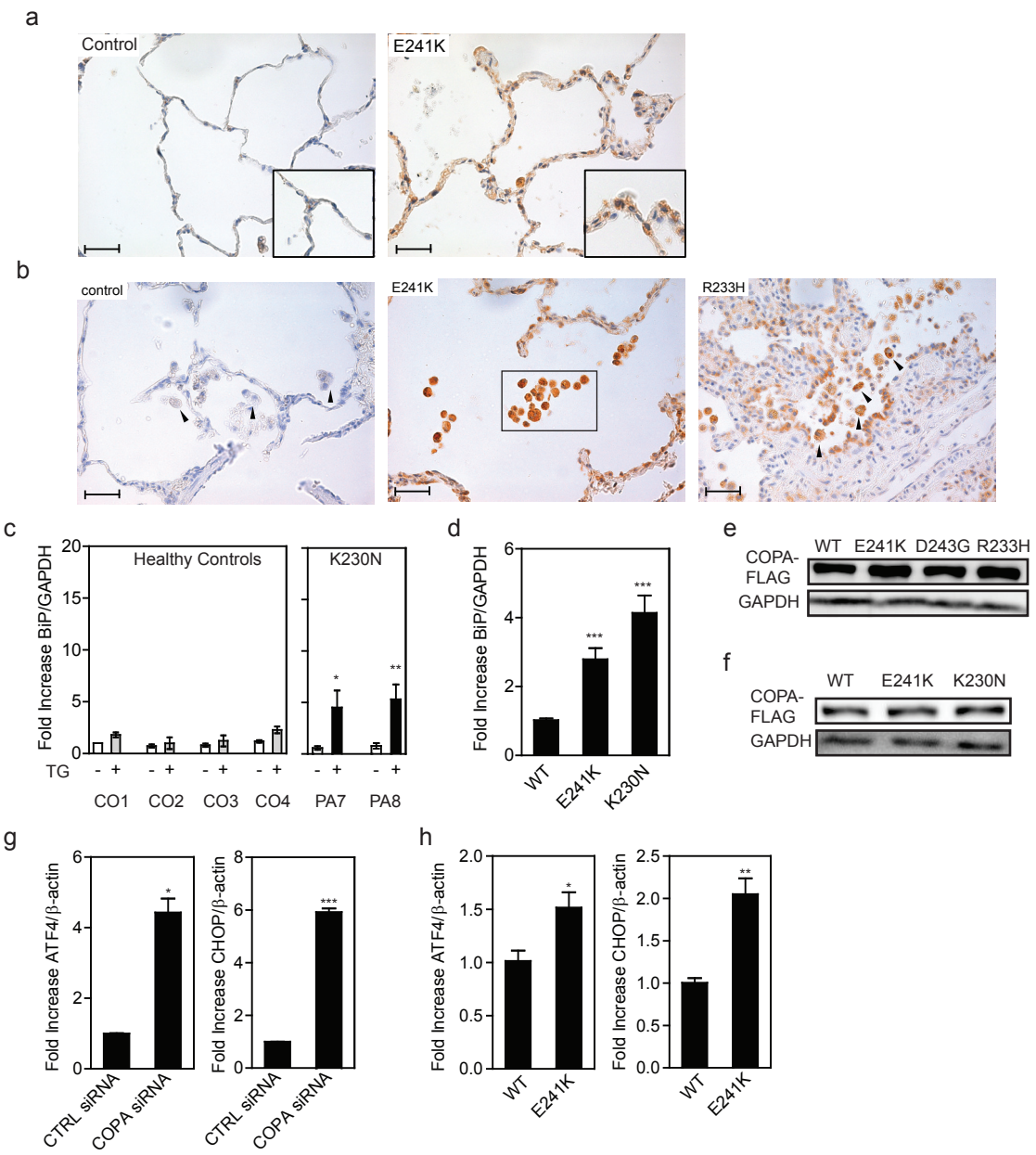
Supplementary Figure 3. COPA expression in patient cells is equivalent to controls (a,b) COPA protein expression in the B-lymphoblastoid cell line (BLCL) of 5 controls (CO3 is D.III.6, CO4 is C.III.10, CO5 is D.III.4), 3 patients of Family C (PA1=C.IV.15, PA2=C.V.1, PA3=C.IV.2), one patient of Family B (PA4= B.III.3) , 2 patients of family A (PA5=A.II.5, PA6=A.III.6) and 2 patients of family D (PA7=D.III.5, PA8=D.I.2) was analyzed. **(a)** Western Blot analysis of COPA and GAPDH is shown. Numbers indicate the relative expression of COPA to GAPDH. **(b)** Unpaired t test with Welch's correction was used for statistical evaluation of protein expression in affected patients compared to controls. ns, not significant (P>0.05). **(c)** COPA mRNA expression in BLCLs analyzed via qPCR is shown. P values are not significant (>0.05) for all comparisons. **(d)** Immunofluorescent staining of BLCLs. DAPI is stained in blue and COPA in red. Colocalization (yellow) of COPA (red) with the endoplasmic reticulum golgi intermediate complex (ERGIC)-53 (green) in single cells is shown. Staining was visualized by using a Zeiss Apotome widefield microscope.

Supplementary Figure 4

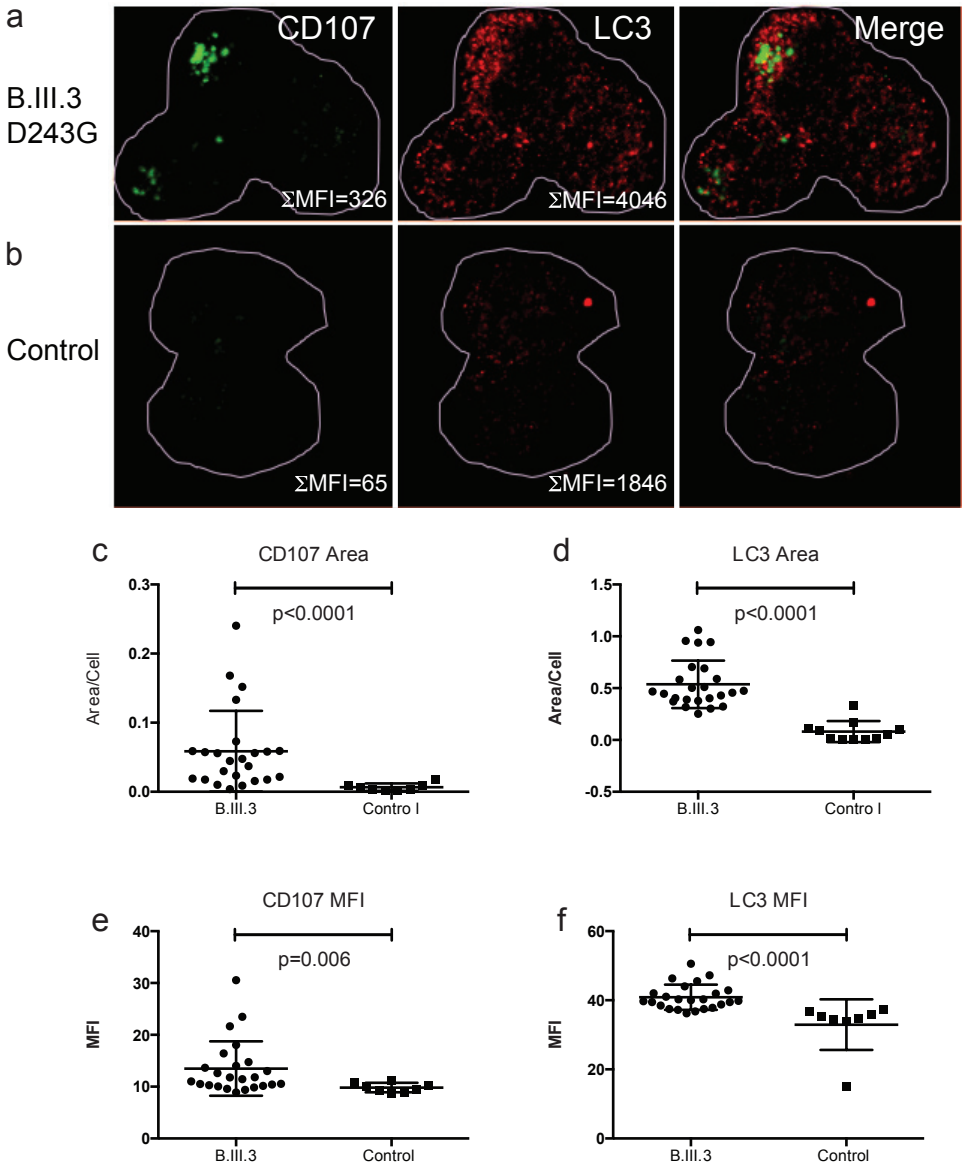


Supplementary Figure 4. Binding of mutant K230N COPA to dilysine motifs is impaired **(a)** Binding of mutant COPA to a dilysine motif was examined by taking either FLAG-tagged wild-type or mutant K230N COPA and incubating it with Wbp1p peptide-coupled agarose beads. The protein-bead complexes were analyzed by western blot (WB) using an anti-FLAG antibody. Numbers indicate the relative expression in percent of wild-type COPA. Statistical analysis of 3 independent experiments is shown. *P<0.05 **(b)** Binding of mutant COPA to a dilysine motif was also assessed within T-REx-293 cells stably expressing FLAG-tagged wild-type or mutant COPA by transiently transfecting cells with an expression construct for GFP-CD8-KKTN. Cell lysates were precipitated with anti-CD8 and immunoprecipitates were assayed for FLAG-COPA expression and GFP-CD8 enrichment. Numbers indicate the relative expression in percent of wild-type COPA or GFP-CD8 expressed in WT COPA cells. Statistical analysis of 5 independent experiments is shown. **P<0.01.

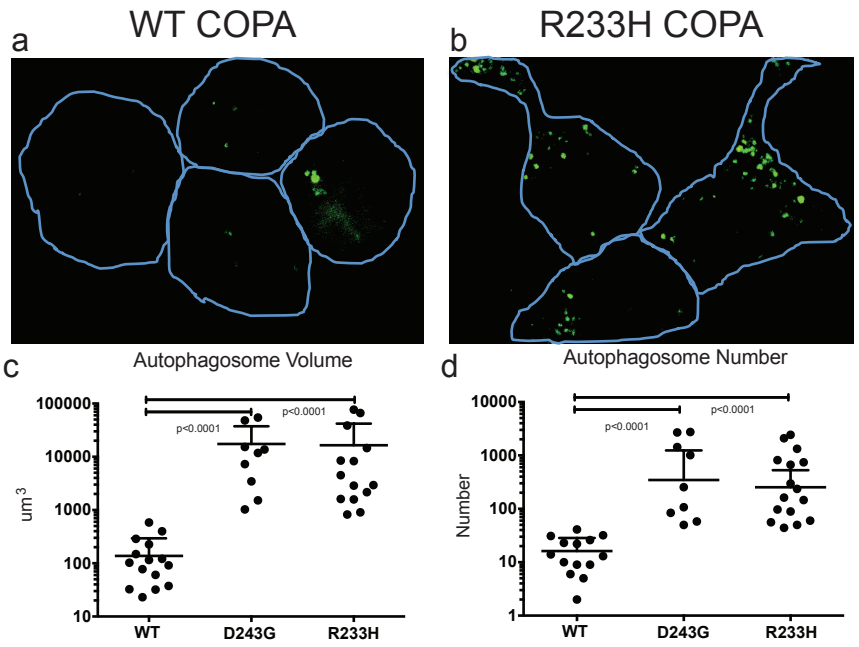
Supplementary Figure 5



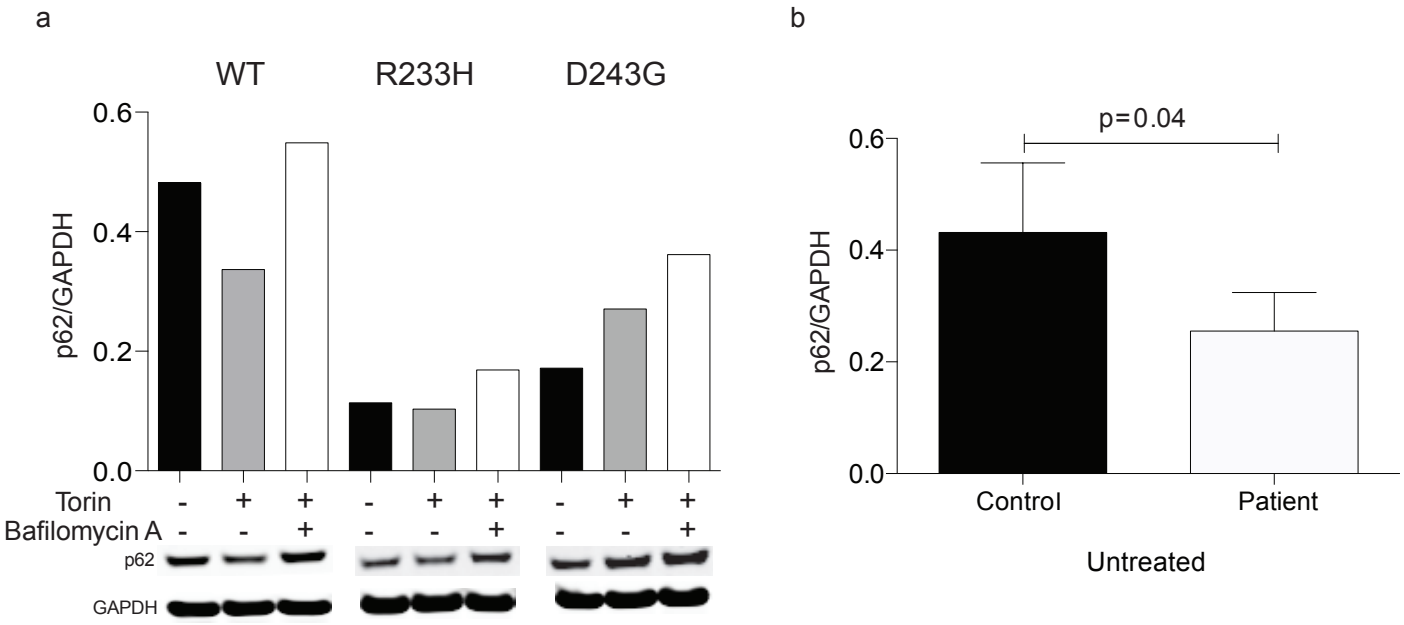
Supplementary Figure 5. Patient lungs or cells demonstrate an increase in ER stress markers *BiP, ATF4 and CHOP* **(a)** Immunohistochemistry stain for the ER stress component BiP in lung biopsy samples from an additional control and an additional E241K patient is shown. Hematoxylin was used for nuclear counterstain. Scale bars indicate 40μm. Enlarged inserts emphasize BiP staining in lung epithelial cells. **(b)** BiP staining in alveolar macrophages is shown (arrows, box). Scale bars indicate 40μm. **(c)** Quantitative PCR analysis of *BiP* expression in untreated (white bars; –) BLCL and treated (grey, black bars; +) BLCL after stimulation with 100mM thapsigargin (TG). Left graph indicates the results from 4 controls (CO 1-4; CO3 is a related control from Family D, D.III.6, and CO4 from family C, C.III.10). All results were normalized to values of untreated control 1. Data shown are from two patients from Family D (PA7-8, D.III.5, D.I.2). One way ANOVA followed by Dunnett's Multiple Comparison Test was used to compare thapsigargin-treated experimental groups with thapsigargin-treated control 1 as a reference. Mean and SEM of one experiment representative of 3 independent experiments is shown. *P<0.05, **P<0.01. **(d)** Graphs demonstrating *Bip* mRNA expression 48 hours after transfection as assessed by qPCR. HEK 293 cells were transiently transfected with wild-type (WT) COPA or mutant E241K or K230N COPA expression constructs. Cells transfected with mutant COPA showed significantly increased *Bip* levels when normalized to cells transfected with wild-type COPA. Mean and SEM are shown for each column. One-way ANOVA followed by Dunnett's Multiple Comparison Test was use to compare experimental groups against wild-type transfected HEK293 cells as the control column. Results shown are from one experiment representative of 3 independent experiments. ***P<0.001. **(e,f)** 293HEK cells were transiently transfected with either WT or mutant FLAG-tagged COPA. Cells were lysed and analyzed by Western Blot for the expression of FLAG-COPA and GAPDH 48h later. **(g)** Induction of ER stress measured by *ATF4* and *CHOP* expression 48h after *COPA* knockdown. Mean and SEM of one experiment representative of 3 independent experiments are shown. Statistical comparisons were made using Welch's unpaired t test. *P<0.05, ***P<0.001. **(h)** Graphs indicating *ATF4* (left) and *CHOP* (right) expression 48h after transfection as assessed by qPCR. HEK 293 cells were transfected with wild-type (WT) or mutant E241K COPA constructs. Mean and SEM of one experiment representative of 3 independent experiments are shown. Statistical comparisons were made using Welch's unpaired t test. *P<0.05, **P<0.01



Supplementary Figure 6. Mutant COPA increases autophagosomes. (a,b) BLCL from patient PA4 (B.III.3) and a control were stained with anti-CD107a (green) or anti-LC3B (red) and imaged in super-resolution using a time-gated STED microscopy. White lines indicate edges of individual cells. **(c,d)** The area of the resulting structures were graphed and compared statistically using the Mann Whitney U test and was found to be significantly increased in patient cells. ***P<0.001. **(e,f)** As an overall measure of cellular autophagosome content, the amount of both LC3 and CD107a was assessed by mean fluorescence intensity (MFI) measurement and was increased in COPA patient BLCLs (LC3B MFI 41 ± 3.6 N=24 vs. 32.9 ± 7.3 N=8, ***P<0.001; CD107a MFI 9.8 ± 0.9 N=8 vs 13.5 ± 5.2 N=24, **P<0.01)

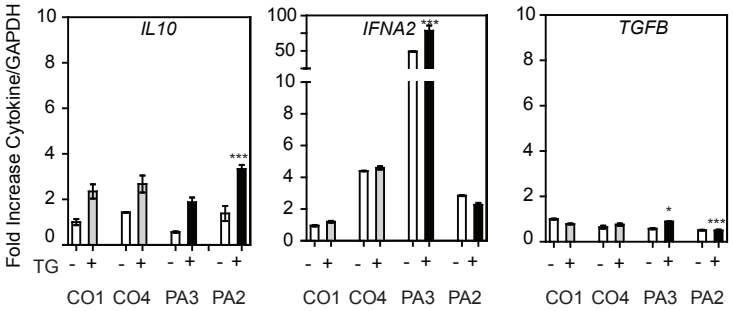


Supplementary Figure 7. Overexpression of mutant COPA increases autophagosome size and number. 293T cells stably expressing GFP-LC3B as an indicator of autophagosomes were transfected with WT, mutant R233H or D243G COPA constructs and GFP puncta were measured for both number and volume using confocal imaging as an indication of autophagosome formation. **(a,b)** Representative pictures are shown for WT and R233H COPA. Blue lines indicate edges of individual cells. **(c)** In quantitative analyses across repeated experiments the expression of D243G and R233H mutant COPA resulted in a statistically significant increase in autophagosome size (WT 138 ± 38 N=17, D243G 17411 ± 6658 N=9, R233H 16467 ± 6797 N=14) and **(d)** autophagosome number (WT 16.2 ± 3.2, D243G 936 ± 372, R233H 582 ± 188). The Mann Whitney U test was used for statistical analysis. ***P<0.001.



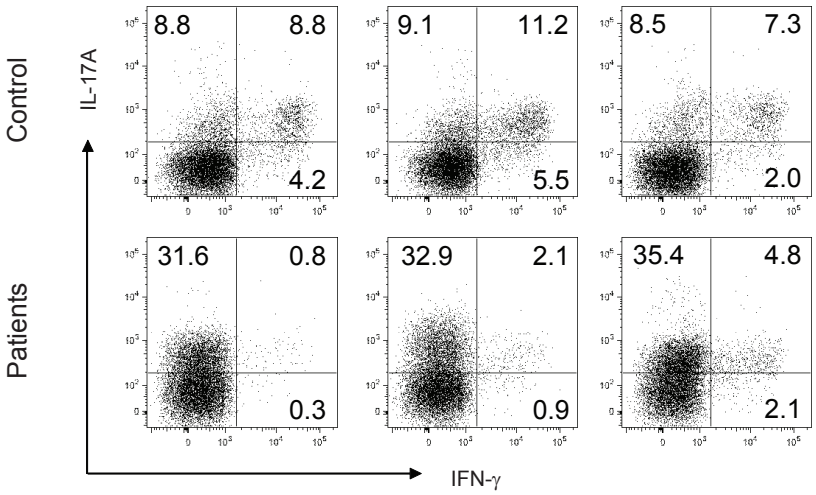
Supplementary Figure 8. Autophagy catabolism is impaired in COPA patient cells. (a) Representative patient or control BLCL were either untreated (black), stimulated with the autophagy-inducing drug Torin (gray), or a combination of Torin and baflomycin A (white bar) to pause autophagy by preventing fusion with the lysosome for four hours. Cells were lysed and evaluated by Western blot analysis for p62 levels (representative example provided). The levels of p62 were quantified using ImageJ software, normalized to GAPDH levels and graphed above the western blot. (b) Student's unpaired t test was used for statistical analysis of untreated control (n=4 black bar) and patients (n=4 white bar) samples. Graph shows mean and SD from pooled data of 3 independent experiments. *P<0.05.

Supplementary Figure 9



Supplementary Figure 9. Immune cell cytokine expression in patient BLCLs. Cytokine expression was measured in BLCLs from CO1, CO4 (C.III.10), PA2 (C.V.1) and PA3 (C.IV.2) either untreated (white bars; –) or treated (grey, black bars; +) with TG. Samples were analyzed via qPCR for mRNA levels of *IL10, IFNA2, and TGFB*. Shown are normalized mRNA levels against the average value for healthy control 1 (CO1) in the absence of stimulation. Errors bars indicate the standard error for triplicates in the assay. Results are representative of 3 independent experiments. Nothing indicated P>0.05; *P<0.05; ***P<0.001.

Supplementary Figure 10



Supplementary Figure 10. IFNγ$^+$IL17$^+$CD4$^+$ T cells in COPA patients versus controls. CD4$^+$ T cells from three COPA patients and three age-matched controls were analyzed for intracellular cytokines IL-17A and IFN-γ after stimulation with PMA and Ionomycin. FACS plots gated on living CD3+CD4$^+$ T cells are shown.